Article

Phenotyping of corn plants with effect of mesotrione herbicide

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Abstract

Mesotrione is an herbicide that is used for the control of a wide spectrum of weeds during pre- and post-emergence in the crop of corn (*Z. mays* L). The objective of the present study was to evaluate the effects of mesotrione on growth, pigmentation, with images in the visible spectrum (red, green, and blue, RGB) and fluorescence (Fv/Fm) in the corn cultivars Cacahuacintle, HS-2 and Vitamaíz. The plants were grown in greenhouses, in the College of Postgraduates, Montecillo *Campus* during 2020, they were analyzed in the vegetative stage V3-V4 with a design of randomized complete blocks and with factorial arrangement. The treatments evaluated were controls (water and adjuvant without mesotrione) and mesotrione 1X and 2X. Ten days after application, images of the plants were obtained, which were analyzed with the LemnaGrid program. None of the mesotrione doses altered the growth of the cultivars, although there were effects on the color of the plants. The loss of green color (chlorosis) occurred in more than 50% of the base of the leaf blade and with spots at the apex. The images of chlorophyll fluorescence and the Fv/Fm index in leaf fragments indicated that the highest dose of mesotrione (2X) in the cultivars Cacahuacintle and Vitamaíz maintained values similar to the controls; in contrast, in HS-2 those values decreased. The results of the present study demonstrated the usefulness of non-invasive phenotyping with RGB images, and of the chlorophyll fluorescence to evaluate the effect of herbicides on crops.

Keywords: fluorescence, mesotrione, pigmentation, RGB.

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Introduction

Corn (*Z. mays* L.) is the second most consumed cereal in the world after wheat. The United States produce approximately 41% of the world's total, China 20%, and Brazil 6% (FAO, 1993). Mexico ranks seventh with a contribution of around 27 169 t and an average annual yield of 3.8 t ha^{-1} (Ruiz *et al*., 2019). Losses in crop yields from weeds are variable, but can be total, which is why the use of herbicides has increased significantly (Aktar *et al*., 2009). Among the most commercialized, mesotrione [2-(4-methylsulfonyl-2 nitrobenzoyl) 1,3-cyclohexanedione] is used to control monocot and dicot weeds (Ulguim *et al*., 2013).

The compound belongs to the chemical family of triketones; its action consists of the inhibition of the 4-HPPD enzyme, which interrupts the biosynthesis of carotenoids and generates whitish lesions, chlorosis and finally necrosis (Mitchell *et al*., 2001). The technical sheet of mesotrione indicates that 'it is highly toxic to aquatic organisms, although maintaining adequate management conditions, ecological problems should not be expected, with medium to high mobility in soil and low accumulation potential'. However, studies of its effects have shown the high potential risk of groundwater contamination and intermediate potential risk of surface water contamination.

Currently, the presence of herbicide residues has been observed in soils and waters in different countries at higher levels than allowed (Echeverría-Sáenz *et al*., 2012; Lopez *et al*., 2015; Zheng *et al*., 2016; Herrero-Hernández *et al*., 2017). *Amaranthus tuberculatus* (*var* Rudis), as a weed in corn and soybean crops, required doses of 420 g ai. ha^{-1}, several times higher than the recommended 105 g ai. Ha^{-1}; this showed that the species has acquired resistance to the herbicide (Oliveira *et al*., 2018). However, knowledge of the effects of herbicides on corn plants is scarce; the effects of these compounds on the stability of photosynthetic pigments or on photosynthetic capacity have not been documented with non-invasive methods.

Red, green, and blue (RGB) images are used in phenotype analysis methods; these are not expensive and allow the simultaneous analysis of numerous specimens, so they are recognized as high-throughput studies (Halpering *et al*., 2017). In addition to these methods, sensors that quantify chlorophyll fluorescence by means of pulse-amplitude modulation (PAM) are now used, which, at the same time, allow evaluating alterations in photosynthesis and the quantum efficiency of photosystem II with the Fv/Fm index (Rosseau *et al*., 2013).

Platforms for phenotype analysis allow the generation of hundreds of images that, with certain programs, quantify phenotypic traits, such as those of growth and color changes associated with loss of pigmentation in leaves (Klukas *et al*., 2014; Thomas and Ougham, 2014). The latter, with the name of stay green, is a trait associated with yield in standard conditions and conditions of stress due to heat or drought (Thomas and Ougham, 2014; Cayetano *et al*., 2021).

Among the programs for phenotype analysis are those cited by LemnaGrid (Kuklas *et al*., 2014), CVPlant (Gehan *et al*., 2017); Phenotiki (Minervini *et al*., 2017); OpenStart (Haselimashhadi *et al*., 2020), which process databases of hundreds of images (Rousseau *et al*., 2013; Feng *et al*., 2018) with fluorescence (FLUO) (Jansen *et al*., 2009), infrared (INFRA) (Prashar and Jones, 2016) and multispectral (Chivasa *et al*., 2020) sensors. The objective of the study was to evaluate the effects of 1X and 2X doses of the mesotrione herbicide on the phenotype and physiology of the corn cultivars: Cacahuacintle, HS-2 and Vitamaíz. Establish the bases for the identification of damage caused by herbicides in corn cultivars with non-invasive, low-cost methods and with a high number of repetitions in the field or greenhouse.

Materials and methods

The study included the corn cultivars: Cacahuacintle, HS-2 and Vitamaíz. The Postgraduate Program in Genetic Resources and Productivity of the College of Postgraduates donated the cvs. Cacahuacintle and HS-2. The Center for Research and Advanced Studies of the National Polytechnic Institute, Irapuato Unit donated the *cv* Vitamaíz. The research was carried out in facilities of the Postgraduate Program in Botany of the College of Postgraduates, Montecillo *Campus*, Mexico (High Valleys, 19° 29' north latitude, 98° 53' west longitude and 2 250 m altitude).

The seeds of the three cultivars were sown in plastic pots, with 1.2 kg of plant peat (Pro-Mix®). Each pot contained a plant and irrigation with Steiner's nutrient solution (Steiner, 1984) was applied, at field capacity, until the V3 stage, with the third leaf developed. The plants were distributed in an experimental design in randomized complete blocks with a factorial arrangement (A × B), where the first factor (A) represented the mesotrione herbicide (Callisto® 480 SC, Switzerland) and adjuvant (mineral oil); the second factor (B) was made up of the cultivars of corn.

The treatments were represented by cultivars and mesotrione doses. Factor A included three levels, exemplifying the treatment with adjuvant, and treatments in 1X and 2X doses of the herbicide. Factor B included three levels or cultivars; and as additional treatments, the controls with water were included for each cultivar. The experimental unit was one plant per pot and four repetitions per treatment.

The dilutions were prepared according to the manufacturer's instructions, 1 ml of mesotrione + 1 mL of adjuvant, which corresponds to 1X or 80 g of ai. ha^{-1} and double for the 2X concentration. Both concentrations were completed with water, to a final volume of 1 L (v/v). The treatments were applied with a high-pressure plant sprinkler (Lalatech®), with TeeJet® XR 11003 flat fan nozzle (USA), calibrated to release 1.5 L, pressure of 2.5-3 bar and 40 cm high with respect to the plant.

The phenotype of the plants was evaluated in the vegetative stage V3-V4, ten days after applying the herbicide, with the Scanalyzer PL imaging system (LemnaTec). Each pot was placed in the platform cabin, illuminated with white light lamps, and images were obtained with the top view and side view cameras in the visible region of the RGB spectrum (400-700 nm). The camera resolution of the Scanalyzer PL (Baster AG, Ahresburg Germany) generated images with 1 628 x 1 236 pixels, and 4.4 x 4.4 μm per pixel. A total of 192 images were analyzed with the LemnaGrid program (Figure 1), which, by means of algorithms, debug the image for the extraction of information through a grid.

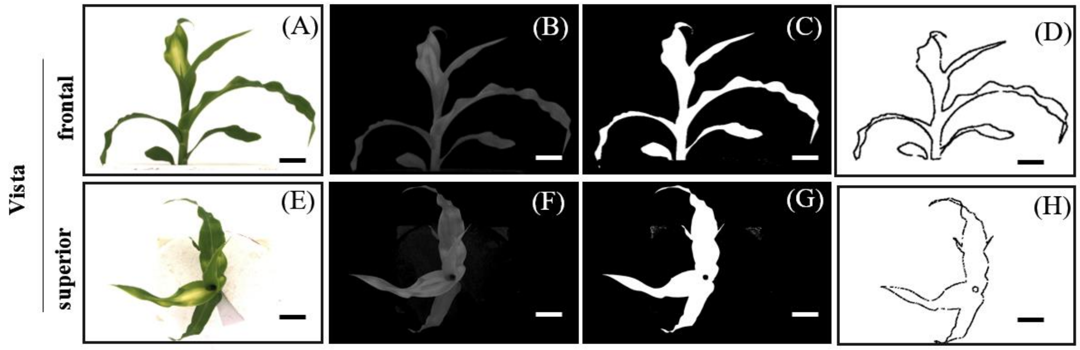

Figure 1. Example of front (A) and top (E) view images of corn plants processed to evaluate morphometric parameters. The background of the image is separated (B-F) from the image, is transformed to binary in black and white (C-G) and the area is delimited (D-H). The bar represents 3 cm.

The program separated the foreground and background from the original image of the top and side views. With each image, the digital quantitative biomass or projected area was calculated, which corresponded to the volume per pixel, which is an approximation to the total biomass (fresh weight) of the plant; in addition, other variables are obtained with the images, which are called convex hull area (cm^2), caliper length (mm) and compactness, which allows the calculation of the index that generates the polygon that forms the edge of the plant divided by the projected area (Jansen *et al*., 2009).

The top view of the images of the leaf blade, the toxicity of the herbicide in the plants was determined, by quantifying the symptoms such as chlorosis and whitish lesions. The program classified the colors by removing the background, categorizing healthy photosynthetic tissue with a green color, the tissue affected by the herbicide with yellow, and produced binary images, with which the senescence of the leaves was quantified.

In addition, the photosynthetic capacity was evaluated by means of the photochemical efficiency of photosystem II (PSII). This by means of the Fv/Fm index, where Fv is the variable fluorescence of chlorophyll (Fm-F0), Fm is the maximum fluorescence and F0 the initial fluorescence. The difference between the fluorescence generated with the first stimulus with light and the maximum stimulation with light is the fluorescence emitted when electrons are used in photosynthesis by 'quantity' of light absorbed (Goltsev *et al*., 2009). For these evaluations, fragments of 16 cm^2 of the central region of the leaf or areas that showed the toxic effects of the herbicide were used.

Initially, the leaf fragments were kept in darkness for 30 min and introduced into an enclosed chamber (PhotoSystem Instruments Open FluorCam FC-800, Czech Republic) to quantify fluorescence with the FluorCam program (version 7), configured for a reading with the Kaustky curve and constant light pulses. The values of F0 and Fm were obtained together with the images with the fluorescence of the leaves. The results were analyzed with Anova and Tukey's multiple comparison of means ($p \leq 0.05$), with the statistical programs Minitab® 19 and SAS® 13. The figures were processed in GraphPad Prism8.

Results and discussion

The effect of mesotrione on plants of the three corn cultivars was evaluated with the phenotyping platform and the LemnaGrid program in 384 images acquired in top and side views. Initially, the separation of the foreground and background was performed on original images (Figure 1 A-E), transformed into grayscale (Figure 1 B-F) and later into binary images in black and white (Figure 1 C-G), which were segmented into lines (Figure 1 D-H).

The result is shown in images, in front view, of a representative plant, projected area (Figure 2A), convex hull area (Figure 2B), compactness (Figure 2C) and caliper length or maximum length (Figure 2D). These variables have been used in phenotyping studies of plants with abiotic stress (Kuklas *et al*., 2014) that demonstrated their usefulness to evaluate productivity indices. This was the case of a study in sorghum (*Sorghum bicolor* and *Sorghum sudanense* (Piper) Stapf.) that demonstrated the negative effect on growth due to the restriction of nitrogen in the soil (Neilson *et al*., 2015).

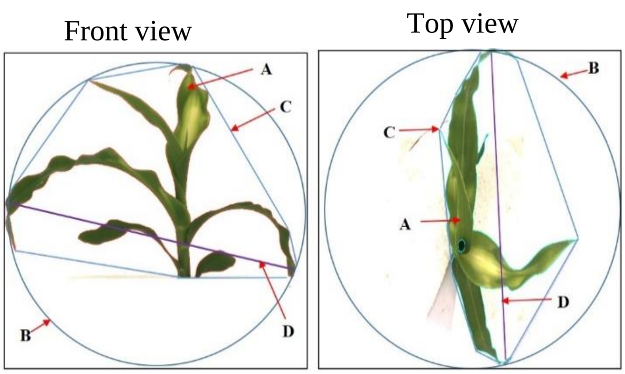

Figure 2. Example of front and top view images of corn plants and morphometric variables generated with the LemnaGrid program. Projected area (A); convex hull area (B); compactness (C); and caliper length in the front view (D).

Phenotype assessment was also described in barley (*Hordeum vulgare* L.), *Arabidopsis* (Acosta Gamboa *et al*., 2016) and beans (*Phaseolus vulgaris* L.) with moisture deficit stress (Padilla-Chacón *et al*., 2019). This study showed that non-destructive phenotyping is a useful tool for the evaluation of plants with herbicide damage.

In the quantitative analysis of morphometric variables, in the images with the top view it showed no significant differences between treatments (Figure 3). This indicated that the mesotrione herbicide did not negatively affect the development and growth of the three cultivars. However, in the front view images, differences were observed between cultivars regardless of the sprayed compounds.

These observations indicated that, in the variables of projected area, convex hull area and caliper length in the *cv* Cacahuacintle, they were between 10 and 15% higher than in the cultivars HS-2 and Vitamaíz (Figure 3) and that the latter was 15% more compact than the other two cultivars. The images with the top view did not show significant differences in the variables of projected area, convex hull area and caliper length, except in compactness, which, according to the statistical analysis, the growth of HS-2 and Vitamaíz was lower, between 5 and 7%, compared to the Cacahacintle cultivar (Figure 3).

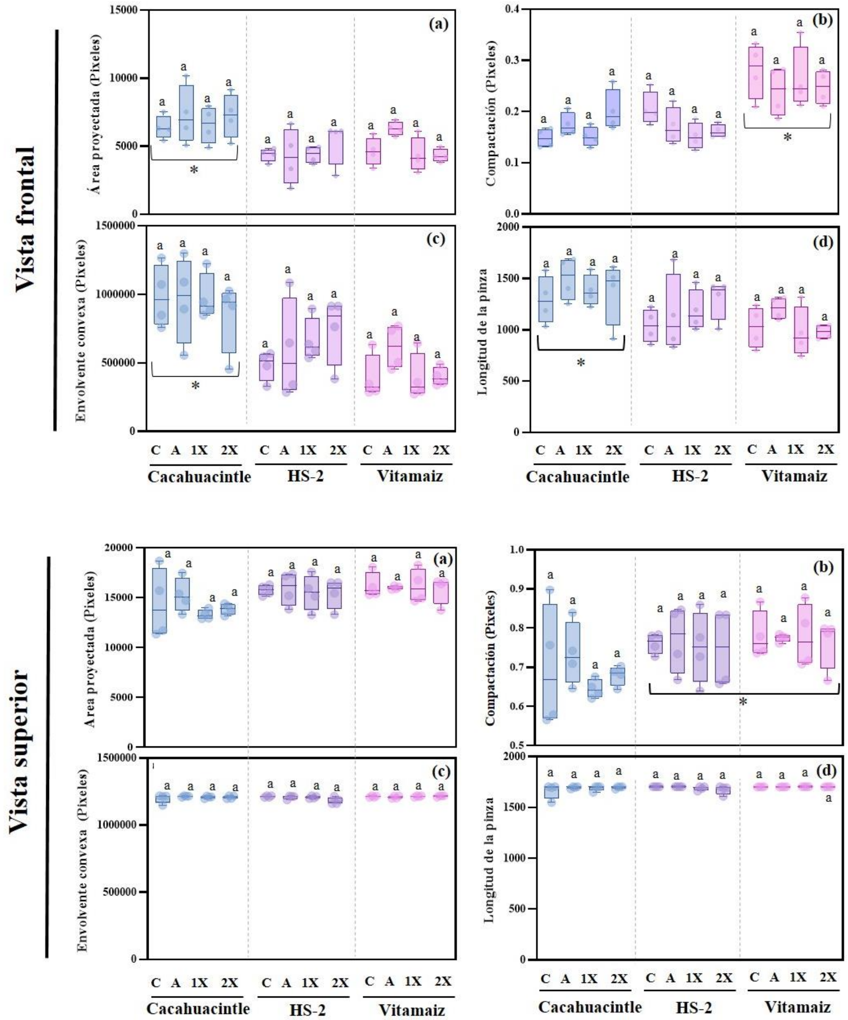

Figure 3. Effect of mesotrione herbicide, 10 days after its application in commercial doses (1X) and double doses (2X), on morphometric growth variables of plants of Cacahuacintle, HS-2 and Vitamaíz corns. Digital area (a), compactness (b), convex hull (c) and caliper length (d), water (C) and adjuvant (A). Different letters on the bars, in each cultivar, indicate significant differences ($p \leq 0.05$). *= below the bars they indicate significant differences between cultivars (n= 4).

The results of the present study coincide with those reported by Bibi *et al*. (2019). These authors evaluated corn plants treated with the atrazine herbicide, they do not show alterations in growth, even with doses higher than recommended, although the herbicide did not alter growth, the authors demonstrated that, in the leaves, the concentrations of soluble sugars and proline did increase. In another study, using analysis of morphometric variables, the effect of atrazine and mesotrione was compared in corn cultivars in post-emergence and weeds, such as *Chenopodium album* L., *Polygonum pensylvanicum* L. and *Capsella bursa-pastoris* L. (Creech *et al*., 2004).

They also indicate that the study showed that none of the herbicides altered the accumulation of dry biomass of corn plants in the stem (Creech *et al*., 2004). The partial results of the present study and those already mentioned suggest that corn plants have mechanisms of tolerance or elimination of herbicides that prevent growth from being altered.

Information on the degradation mechanisms of herbicides that allow corn plants to avoid the effects of herbicides is scarce. However, it was found that, in corn plants, the paraquat stimulated the mitogen-activated protein kinase signaling cascade, which has a role in the stress signaling pathway, which is mediated by abscisic acid (ABA) associated with MAPK and MAPK5 (Ding *et al*., 2009).

Another study showed that certain treatments with the benzyladenine cytokinin promoted decreased paraquat toxicity in corn plants (Durmuş and Kadioğlu, 2005). Due to the lack of any effect of mesotrione on the growth of corn plants of the three cultivars in the present study, the presence of a mechanism of detoxification of the herbicide, similar to those documented in other studies with other herbicides, is not ruled out. Therefore, the continuation of the present study will include biochemical evaluations focused on the knowledge of the response mechanisms of young corn plants to mesotrione.

From images with the top view, the loss of green color due to the effect of mesotrione was evaluated with the LemnaGrid program. For this, the green (healthy) and yellow (chlorosis) color of the image was segmented in percentage (Figure 4A). The results showed that the loss of green color in the three genotypes was 10 to 15% in the corn blade with the 1X dose of mesotrione. The 2X dose increased the symptoms of loss of green color; the effect was observed with greater variability in the cultivars Cacahuacintle and HS-2. These cultivars showed loss of 30% of the green color and in Vitamaíz it was 20% (Figure 4B, 4C).

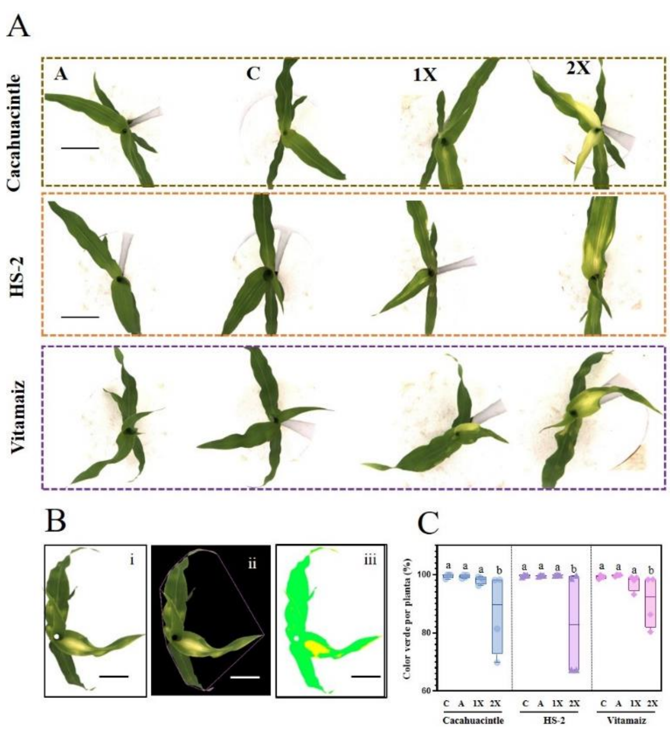

Figure 4. A) example of top view images of the cultivars Cacahuacintle, HS-2 and Vitamaíz after 10 days of receiving 1X and 2X doses of mesotrione and controls. B) example of top view images analyzed in the LemnaGrid program; i) original image, ii) morphometric variables and iii) color segmentation. C) graph of the average (± se) in percentage of green color of corn plants of three cultivars. A) water, C) adjuvant, 1X) recommended commercial dose and 2X) double dose. The bar represents 3 cm. Different letters on the bars, in each cultivar, indicate significant differences ($p\leq 0.05$), (n= 4).

The level of mesotrione damage in the third leaf, with the adaxial side frontally exposed, was assessed with the LemnaGrid program (Figure 5). Although the young leaves cover the lower ones in the top view images, the results showed that the loss of green color with both doses represented between 40 and 50% in the three cultivars. However, segmentation analysis showed that the loss of green color was different along the blade, since the largest loss, between 60 and 75%, of color occurred in the middle of the leave from the base (Figure 5). In contrast, the loss of green color in the apical half was between 15 and 30% (Figure 5) and with some areas with damage. This result indicated that the herbicide mainly affects the young tissues of the blade.

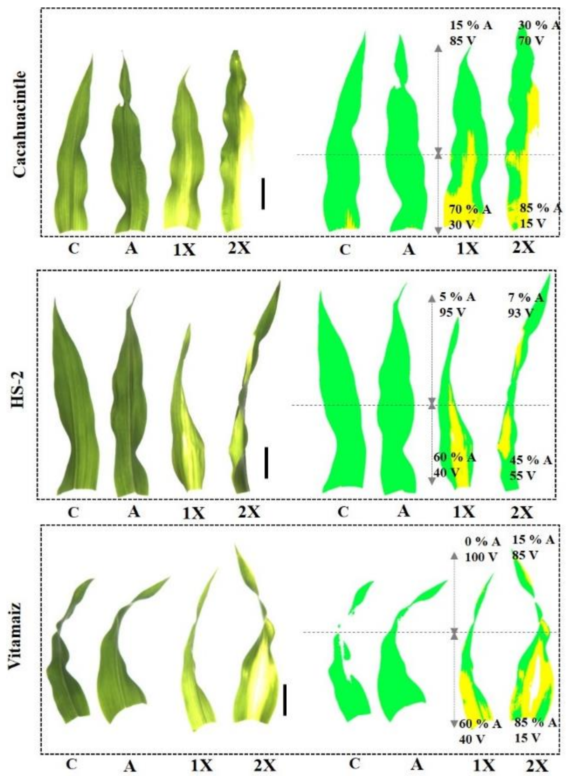

Figure 5. Effect of mesotrione herbicide on blades of the corn cultivars; Cacahuacintle, HS-2 and Vitamaíz (C: adjuvant and A: water), mesotrione 1X and 2X separated into green (healthy) and yellow (chlorosis) segmentation. Left (original images) and right (analyzed with the LemnaGrid program). The bar represents 2 cm. Dotted lines indicate the basal and apical region of the blade. V= green, A= yellow.

There are currently no reports on the distribution of herbicide damage in corn plant blades. Anatomical changes related to green color loss have not been documented either. The results of the present study showed that corn plants significantly decreased their photosynthetic capacity and inhibited the synthesis of photosynthetic pigments, accelerated their degradation, in specific areas of the blade dependent on the age of the tissue and growth was not altered by the effect of toxicity with mesotrione.

Wang *et al*. (2019) proposed a method that replaces the evaluation by the normalized difference vegetation index or NDVI, in the complete leaves of corn plants by means of images analyzed with algorithms that allow identifying the deficiencies in nutrients in the leaves in color scale.

Complete corn plants have been evaluated in the field with methods that combine the use of other sensors of NDVI (RGB and NIR), chlorophyll content index (SPAD) and color segmentation, they have been analyzed with the aim of evaluating numerous cultivars in a short time (Wu *et al*., 2015).

The qualitative results of the Fv/Fm index of the three cultivars showed that, in the control plants, sprayed with water and with adjuvant, the red color predominated in most of the tissue with values of Fv/Fm equal to 0.8 (Figure 6A). With the 1X dose of mesotrione, the red color decreased and changed to yellow green in the three cultivars, according to the color scale represented with Fv/Fm values between 0.6 and 0.7, thus it was possible to detect differences in the proportion of yellow and green color. The *cv* HS-2 decreased the yellow color almost completely and changed to green; in contrast, in the cultivars Cacahuacintle and Vitamaíz, the yellow color was maintained only in some regions of the leaf.

With the 2X dose, the leaf fragments maintained the effects that at the 1X dose, even with a slight increase in yellow color. The results were quantitatively validated with the FluorCam7 program (Figure 6B), using this method, it was determined that the average values of Fv/Fm did not show significant differences in the Cacahuacintle cultivar in 1X and 2X doses with respect to controls. This result coincided with the highest yellow color in the images (Fv/Fm= 0.7). In contrast, in the cultivars HS-2 and Vitamaíz, a decrease in the values of Fv/Fm to 0.6 and 0.7 was observed, in the images the yellow color had less color intensity (Figure 6B).

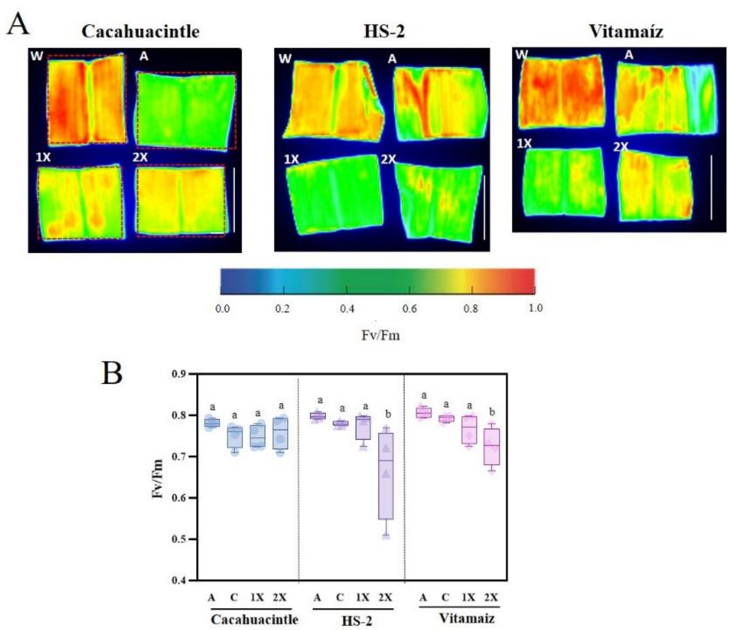

Figure 6. A) images of chlorophyll *a* fluorescence in fragments of the third leaf of corn plants of the three cultivars Cacahuacintle, HS-2 and Vitamaíz, control (water and adjuvant), mesotrione 1X and 2X. The bar represents the color scale showing the quantum efficiency of photosystem II (Fv/Fm) in values from 0 to 1. B) average (±se) of the quantum yield of photosystem II (Fv/Fm) of leaf fragments. Different letters on the bars, in each cultivar, indicate significant differences ($p \leq 0.05$). (n= 4).

Among the variables most commonly used for plant phenotyping, chlorophyll fluorescence has advantages because its measurement is fast and non-invasive (Feng *et al*., 2018). The results obtained in this study indicated that the images in RGB and the values of Fv/Fm showed that mesotrione did not affect the photosynthetic capacity of the cultivars Cacahuacintle and Vitamaíz, in contrast, in the cv. HS-2, the Fv/Fm index decreased.

The differences in the values of Fv/Fm due to the effect of mesotrione between the three cultivars may be due, in part, to the mechanisms of formation/elimination of reactive oxygen species (ROS) in leaf tissues. This aspect was presented in a study of the effect of glyphosate on transgenic corn plants tolerant to the herbicide (Feng *et al*., 2013). Chlorophyll fluorescence images exhibited heterogeneity in leaf response to herbicide and temporal variation in the whole plant. The results of this study also showed that mesotrione damage varies in intensity and between the areas of each leaf; which demonstrated the greater effect of the herbicide on younger leaf tissues.

The methods used in the present study allow the development of studies with a greater number of plants (cultivars) and chemical products (herbicides), which provide reliable information on the susceptibility or tolerance of a species or cultivar to this type of stress, which could be used as a parameter for screening at low cost in a greater number of cultivars or genetic material, in addition, determine when a species is tolerant or susceptible to some type of herbicide.

Conclusions

The symptoms of mesotrione chlorosis in 1X and 2X doses do not alter the phenotype or growth variables of the plants of the three corn cultivars. The symptoms in the leaf blades are heterogeneously distributed, are greater at the base and irregular towards the apex. The chemical efficiency of photosystem II is dependent on the cultivar.

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Cited literature

Acosta, G. L. M.; Liu, S.; Langley, E. X.; Campbell, Z.; Castro, G. N. and Mendoza, C. D. 2016. Moderate to severe water limitation differentially affects the phenome and ionome of Arabidopsis. Funct. Plant Biol. 44(1):94-106.

Aktar, M. W.; Sengupta, D. and Chowdhury, A. 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol. 2(1):1-12.

Bibi, S.; Khan, S.; Taimur, N.; Daud, M. K. and Azizullah, A. 2019. Responses of morphological, physiological, and biochemical characteristics of maize (*Zea mays* L.) seedlings to atrazine stress. Environ. Monit. Assess. 191:717.

Cayetano, M. M.; Peña, Valdivia, C. B.; García, E. A.; Jiménez, G. J.C.; Galván, E. I. G. and Padilla, C. D. 2021. Humidity restriction, high night temperature and their combination, during post flowering on common bean (*Phaseolus vulgaris* L.) canopy and pod senescence. Legume Res. Doi: 10.18805/LR-592.

Chivasa, W.; Mutanga, O. and Biradar, C. 2020. UAV-Based multispectral phenotyping for disease resistance to accelerate crop improvement under changing climate conditions. Remote sensing. 12(15):2445.

Creech, J. E.; Monaco, T. A. and Evans, J. O. 2004. Photosynthetic and growth responses of *Zea mays* L. and four weed species following post-emergence treatments with mesotrione and atrazinet. Pest Manag Sci. 60(11):1079-1084.

Ding, H. D.; Zhang, X. H.; Xu, S. C.; Sun, L. L.; Jiang, M. Y.; Zhang, A. Y. and Jin Y. G. 2009. Induction of protection against paraquat induced oxidative damage by abscisic acid in maize leaves is mediated through mitogen activated protein kinase. J. Integr. Plant Biol. 51(10):961-972.

Durmuş, N. and Kadioğlu, A. 2005. Reduction of paraquat toxicity in maize leaves by benzyladenine. Acta Biol Hung. 56(1-2):97-107.

EcheverrÌa, S. S.; Mena, F.; Pinnock, M.; Ruepert, C.; Solano, K.; Cruz, E.; Campos, J.; Sánchez, A. B.; Lacorte, S. and Barata, C. 2012. Environmental hazards of pesticides from pineapple crop production in the Río Jiménez watershed (Caribbean Coast, Costa Rica). Sci. Total Environ. 440:106-114.

FAO. 1993. Organización de las Naciones Unidad para la Alimentación y la Agricultura. El maíz en la nutrición humana. Colección FAO: Alimentación y nutrición Nº 25. Roma Italia. http://www.fao.org/3/t0395s/T0395S02.htm#Tipos%20de%20maiz.

Feng, X.; Yu, C.; Chen, Y.; Peng, J.; Ye, L.; Shen, T. 2018. Non-destructive determination of shikimic acid concentration in transgenic maize exhibiting glyphosate tolerance using chlorophyll fluorescence and hyperspectral imaging. Front. Plant Sci. 9(468):1-16.

Gehan, M. A.; Fahlgren, N.; Abbasi, A.; Berry, J. C.; Callen, S. T.; Chavez, L.; Doust, A. N.; Feldman, M. J.; Gilbert, K. B.; Hodge. J. G.; Hoyer, J. S.; Lin, S.; Liu, S.; Lizárraga, C.; Lorence, A.; Miller, M.; Platon, E.; Tessman, M. and Sax, T. 2017. PlantCV v2: Image analysis software for high-throughput plant phenotyping. Peer J. Dec 1;5:e4088

Goltsev, V.; Zaharieva, I.; Chernev, P. and Strasser, R. J. 2009. Delayed fluorescence in photosynthesis. Photosyn. Res. 101(2-3):217-232.

Halpering, O.; Gebremedhin, A.; Wallach, R. and Moshelion, M. 2017. High-throughput physiological phenotyping and screening system for the characterization of plant environment interactions. The Plant J. 89(4):839-850.

Haselimashhadi, H.; Mason, J. C.; Mallon, A. M.; Smedley, D.; Meehan, T. F. and Parkinson, H. 2020. OpenStats: A robust and scalable software package for reproducible analysis of high-throughput phenotypic data. PLoS One, 15(12):0242933.

Herrero, H. E.; Rodríguez, C. M. S.; Pose, J. E.; Sánchez, G. S.; Andrades, M. S. and Sánchez, M. M. J. 2017. Seasonal distribution of herbicide and insecticide residues in the water resources of the vineyard region of la rioja (Spain). Sci. Total Environ. 609:161-171.

Jansen, M.; Gilmer, F.; Biskup, B.; Nagel, K. A.; Rascher, U.; Fischbach, A.; Briem, S.; Dreissen, G.; Tittmann, S.; Braun, S.; Jaeger, I.; Metzlaff, M.; Schurr, U.; Scharr, H. and Walter. A. 2009. Simultaneous phenotyping of leaf growth and chlorophyll fluorescence via growscreen fluoro allows detection of stress tolerance in Arabidopsis thaliana and other rosette plants. Funct. Plant Biol. 36(11):902-914.

Kuklas, C.; Chen, D. and Pape, J. M. 2014. Integrated analysis platform: an open-source information system for high-throughput plant phenotyping. Plant Physiol. 165(2):506-518.

Lopez, B.; Ollivier, P.; Togola, A.; Baran, N. and Ghestem, J. P. 2015. Screening of french groundwater for regulated and emerging contaminants. Sci. Total Environ. 15(518-519):562-573.

Minervini, M.; Giuffrida, M. V.; Perata, P. and Tsaftaris, S. A. 2017. Phenotiki: an open software and hardware platform for affordable and easy image-based phenotyping of rosette-shaped plants. Plant J. 90(1):204-216.

Mitchell, G.; Bartlett, D. W.; Fraser, T. E. M.; Hawkes, T. R.; Holt, D. C.; Townson, J. K. and Wichert, R. A. 2001. Mesotrione: a new selective herbicide for use in maize. Pest Manag. Sci. 57(2):120-128.

Neilson, E. H.; Edwards, A. M.; Blomstedt, C. K.; Berger, B.; Møller, B. L. and Gleadow, R. M. 2015. Utilization of a high throughput shoot imaging system to examine the dynamic phenotypic responses of a C4 cereal crop plant to nitrogen and water deficiency over time. J. Exp. Bot. 66(7):1817-1832.

Oliveira, M.C.; Gaines, T. A.; Jhala, A. J. and Knezevic, S. Z. 2018. Inheritance of mesotrione resistance in an *Amaranthus tuberculatus* (*var* Rudis) population from nebraska, USA. Front. Plant Sci. 9(60):1-12.

Padilla, C. D.; Peña, V. C. B.; García, E. A.; Cayetano, M. M. I. and Kohashi, S. J. 2019. Phenotypic variation and biomass partitioning during post-flowering in two common bean cultivars (*Phaseolus vulgaris* L.) under water restriction. South Afr. J. Bot. 121:98-104.

Prashar, A. and Jones, H. G. 2016. Assessing drought responses using thermal infrared imaging. *In*: Duque, P. (Ed.) environmental responses in plants: methods and protocols. Springer New York, New York, NY. 209-219 pp.

Rousseau, C.; Belin, E.; Bove, E.; Rousseau, D.; Fabre, F.; Berruyer, R.; Manceau, C.; Jacques, M. A. and Boureau; T. 2013. High throughput quantitative phenotyping of plant resistance using chlorophyll fluorescence image analysis. Plant Methods. 9:17.

Steiner, A. A. 1984. The universal nutrient solution. In: proceedings of the 6th international congress on soilless culture. International society for soilless culture. Wageningen, The Netherlands. 633-649 pp.

Thomas, H. and Ougham, H. 2014. The stay-green trait. J. Exp. Bot. 65(14):3889-3900.

Ulguim, A. D. R.; Perboni, L. T.; Westendorff, N. D. R.; Nohatto, M. A.; Silva, B. M. and Agostinetto, D. 2013. Redução do espaçamento entrelinhas do milho e sua influência na dose do herbicida. Rev. Brazilian Herbicide J. 12(3):232-241.

Wang, X.; Zhang, R.; Song, W.; Han, L.; Liu, X.; Sun, X.; Luo, M.; Chen, K.; Zhang, Y.; Yang, H.; Yang, G.; Zhao, Y. and Zhao, J. 2019. Dynamic plant height QTL revealed in maize through remote sensing phenotyping using a high throughput unmanned aerial vehicle (UAV). Scientific Reports. 9:3458.

Wu, Q.; Sun, H.; Li, M. Z.; Song, Y. Y. and Zhang, Y. E. 2015. Research on maize multispectral image accurate segmentation and chlorophyll index estimation. Guang pu xue yu guang pu fen xi Guang pu. 35(1):178-183.

Zheng, S.; Chen, B.; Qiu, X.; Chen, M.; Ma, Z. and Yu, X. 2016. Distribution and risk assessment of 82 pesticides in Jiulong River and estuary in South China. Chemosphere. 144:1177-1192.